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I, LEANNE MYNOTT, MANAGER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. 2002950658 for a patent by THE CORPORATION OF THE TRUSTEES OF THE SISTERS OF MERCY IN QUEENSLAND as filed on 08 August 2002.

WITNESS my hand this
Nineteenth day of August 2003

LEANNE MYNOTT
MANAGER EXAMINATION SUPPORT AND SALES

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PROVISIONAL SPECIFICATION

for the invention entitled:

"A method of immunomodulation"

The invention is described in the following statement:

A METHOD OF IMMUNOMODULATION

FIELD OF THE INVENTION

The present invention relates generally to a method for modulating the immuno-activity of an antigen-presenting cell and agents useful therefor. More particularly, the present invention relates to a method for preventing or down-regulating one or more functional activities of a dendritic cell. The present invention further provides antibodies and, in particular, monoclonal antibodies, which interact specifically with epitopes present on the surface of dendritic cells, resulting in depletion, down-regulation or destruction of targeted dendritic cell *in vivo* or *in vitro*. The instant invention further provides a method for modulating an immune response in a subject and, in particular, for down-regulating the immuno-activity of an allogeneic immuno-competent graft and/or the immune response of a recipient of a solid organ transplant. The ability to modulate dendritic cell immuno-activity may be useful, *inter alia*, in a range of immuno-therapeutic and immuno-prophylactic treatments that benefit from immune suppression.

BACKGROUND OF THE INVENTION

Reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that this prior art forms part of the common general knowledge in any country.

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Dendritic cells (DC) are potent cellular activators of primary immune responses (Hart, *Blood 90:* 3245-3287, 1997). Immature myeloid DC in non-lymphoid organs react to, endocytose and process antigens and migrate *via* blood and lymph to T cell areas of lymphoid organs. Here, the mature cells present foreign peptide complexed to MHC Class

II to T cells and deliver unique signals for T-cell activation (immuno-stimulation). They also stimulate B lymphocytes and NK cells. DC undergo differentiation /activation during this process, lose their antigen-capturing capacity and become mature, immuno-stimulatory DC that trigger naïve T-cells recirculating through the lymphoid organs. The lymphoid DC subset may have a different migration pathway and although capable of stimulating allogeneic and autologous T-lymphocytes they have been suggested to have a regulatory function (Grouard *et al., J. Exp. Med.* 185: 1101-1111, 1997). As part of the differentiation/activation process, DCs up-regulate certain relatively selectively-expressed cell surface molecules such as the CMRF-44 and CD83 antigens. DC in the thymus and DCs that do not have an activated co-stimulating phenotype probably contribute to central and peripheral tolerance.

Allogeneic transplantation involves the transfer of material from a host to a recipient. In this process, many foreign antigens are introduced into a host and an immune response results when these foreign antigens are detected by the host's immune system. Initially, an immune response involves interactions between the antigen and antigen-presenting cells (APC) such as dendritic cells. *Interstitial* donor DC in heart and kidney contribute to (direct) recipient T lymphocyte sensitization to all antigens but recipient DC, after migrating into the donor tissue, can also stimulate (indirect) alloantigen sensitization of recipient T-lymphocytes. Depletion of heart and kidney and pancreatic islet DC appears to prolong allograft survival. Interestingly, during liver transplantation, donor leucocytes, which may include non-activated dendritic cells, appear to generate allogeneic tolerance. DC are also predicted to contribute to both acute and chronic Graft *Versus* Host Disease (GVHD), the major life threatening complication of allogeneic bone marrow transplantation (BMT). Blood DC counts change during acute GVHD and recent data have suggested that the DC subset constitution of the allogeneic stem cell preparation might relate to GVHD outcome. Recent evidence from a mouse model suggests that host APC contribute to the acute GVHD. DC may in certain circumstance prevent acute GVHD.

Monoclonal antibodies (mAb) which act at the level of the responder T lymphocyte have been investigated as therapeutic immunosuppression agents in allogeneic transplantation.

The CD3 reagent OKT3 *(Orthoclone, Cilag)* is used routinely to treat acute renal allograft rejection. *Campath* 1 (CD52) and its variants have been used in solid organ transplant and BMT. More recent attempts to suppress acute GVHD have involved the antibody ABX-CBL (CD147) (Deeg *et al., Blood 98:* 2052-2058, 2001) and anti-IL-2R mAb Daclizumab (Cahn *et al., Transplantation 60:* 939-942, 1995). Attempts to interfere with the interaction of the responder T-lymphocyte and an APC have focused on antibodies directed against the co-stimulator molecules CD40, CD80 and CD86 or their ligands. Animal studies suggest that blockade of co-stimulator molecules on DC and other APC induces T cell anergy and prolongation of solid organ grafts (Koenen and Joosten, *Blood 95:* 3153-3161; 2000, Kirk *et al., Nat. Med. 5:* 686-693, 1999; Kirk *et al., Proc Natl Acad Sci USA 94:* 8789-8794, 1997). The use of CD80, CD86 and CD28 blocking agents prevents acute GVHD in mice (Blazar *et al., Blood 85:* 2607-2618, 1995) and *in vitro* blockage of allogeneic responses in allogeneic stem cell preparations has been used in clinical BMT with initial encouraging results (Gribben *et al., Blood 87:* 4887-4893, 1996). The use of a reagent that was more selective at targeting differentiated/activated DC might be advantageous.

In humans, at least two populations of DC, the immature myeloid DC and the plasmacytoid DCs, have been identified based on differential expression of CD11c (O'Doherty *et al., J Exp Med 178*: 1067, 1993; O'Doherty *et al., Immunol 82:* 487, 1994) More recent studies have shown that $CD11c^-$ DC have a different phenotype and express higher amounts of CD123, and have a morphology and function distinct from $CD11c^+$ DC (Grouard *et al., J Exp Med. 185:* 1101-1111, 1997). These two subsets are denoted as myeloid lineage $CD11c^+$ DC and plasmacytoid $CD123^+$ DC. It is thought unlikely that there is a direct developmental relationship between them (Robinson *et al., Eur J Immunol 29:* 2769, 1999).

Theoretically, mAb directed at DC administered to the recipient of a solid organ graft would deplete donor DC (i.e. direct) as well as recipient DC (indirect) as they enter the circulation and initiate antigen presentation pathways. Other donor leucocytes may have immunomodulatory capacity. DC depletion therapy might then be ceased after a short

period, allowing tolerance to emerge. Depleting recipient DC may be more efficacious than disrupting co-stimulator pathways. Investigation of this concept has been delayed, however, by the absence of suitable DC reagents. CMRF-44 mAb is an antibody specific for DC and is used for the identification and isolation of human blood DC (Fearnley *et al.*, *Blood 89:* 3708-3716, 1997). The latter authors have shown that the epitope for CMRF-44 mAb (i.e. CMRF-44 Ag) is expressed early in the differentiation of DC from circulating precursor cells.

Given the importance of dendritic cells in the overall immuno-potential of an individual, there is a need to identify agents, which can facilitate modulation of DC activity.

SUMMARY OF THE INVENTION

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

The present invention is predicated in part on the determination that a cell-surface activation molecule may act as a target for agents, the binding of which, results in disablement and/or eventual destruction of the cell. In particular, it has been shown that CMRF-44 mAb is capable of initiating lysis of antigen presenting cells such as DC. More particularly, CMRF-44 is capable of acting as an immuno-suppressive agent, by down-regulating DC function. Thus, the present invention provides reagents useful for the down-regulation of activated DC, and a method for the suppression of an immune response useful *inter alia* for the reduction or prevention of allogeneic graft rejections, graft *versus* host disease, and the amelioration of certain auto-immune inflammatory interactions, such as rheumatoid arthritis.

The present invention, therefore, contemplates a method for modulating the immuno-activity of an antigen-presenting cell (APC) by contacting the APC with an effective amount of an agent which couples, binds or otherwise associates with a cell-surface activation molecule and in turn prevents, inhibits or otherwise down-regulates one or more functional activities of the APC.

Generally, the APC is a DC.

Preferably, the DC is a myeloid DC and, in a particularly preferred embodiment, belongs to the $CD11c^+$ DC sub-population.

In a preferred embodiment, the agent comprises a monoclonal antibody such as, for example, CMRF-44, or a derivative, fragment, homolog, analog or chemical equivalent or

mimetic thereof and the cell-surface activation molecule is a molecule or a derivative, fragment, homolog, analog or chemical equivalent or mimetic thereof, expressed on the surface of a DC and which interacts with CMRF-44 antibody.

The present invention is further directed to a method for modulating an immune response in a subject by administering to the subject an effective amount of an agent which couples, binds or otherwise associates with an antigen presenting cell's surface activation molecule (e.g. a DC surface molecule which interacts with CMRF-44) which in turn prevents, inhibits or otherwise down-regulates one or more functional activities of the APC.

The agent of the present invention may also be used to down-regulate the immuno-activity of an immuno-competent graft such as a bone marrow graft.

Another aspect of the present invention contemplates a method for the prophylactic and/or therapeutic treatment of a condition characterized by the aberrant, unwanted or otherwise inappropriate immuno-activity of an immuno-competent graft by contacting the graft with an effective amount of the agent or a derivative, homolog, analog, chemical equivalent or mimetic thereof which prevents, inhibits or otherwise down-regulates the inappropriate immuno-activity of the graft.

The present invention further extends to pharmaceutical compositions and formulations comprising the agent for use in conjunction with the instant methods, and to the use of such agents in the manufacture of a pharmaceutical composition or formulation.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 are graphical representations showing examples of CMRF-44 expression in cultured human leukocytes. **(A)** PMBC (activated DC are defined as PE^- $FITC^+$ cells, in lower right quadrant), **(B)** purified Lin^- PBMC cultured overnight with GM-CSF and IL-4, **(C)** $CD11c^+$ Lin^- PBMC cultured as in B, and **(D)** $CD123^{hi}$ Lin^- PBMC cultured as in B. In A, the quadrant positions were determined by negative control staining. In B-D, the left hand line represents IgM negative control staining.

Figure 2 are graphical representations showing CMRF-44-specific complement-mediated DC lysis occurs in cultured human PBMC. The combination of CMRF-44 and autologous human serum (AS) deplete $CD83^+$ DC. Treatments = **(A)** AS only, **(B)** CMRF-44 mAb only, **(C)** CMRF-44 + AS, **(D)** negative control IgM + AS. Lower right quadrants show percentage of DC in treated cultured PBMC.

Figure 3 are graphical representations showing Lin^- DC survival is improved with GM-GSF and IL-3 present during overnight culture. **(A)** Cell death analyzed by PI/Annexin-V labeling after overnight culture with or without the addition of GM-CSF/IL-3. **(B)** Example of Lin^- DC, in live forward/side scatter gate showing improved yield of CMRF-44^+ cells after culture with GM-CSF + IL-3 (left-hand curves + IgM negative control).

Figure 4 are graphical representations showing CMRF-44-specific complement-mediated lysis of DC within a cultured purified human DC (Lin^- cell) preparation. The effect on the percentage of $CD11c^+$ HLA-DR^+ cells (dot plots, upper right quadrants) and on the percentage of dead 7-AAD^+ cells (histograms) after treatment with **(A)** medium alone, **(B)** 1:2 v/v AS alone, and **(C)** 20 ug/ml CMRF-44 and AS combined is shown.

Figure 5 are graphical representations showing examples of CMRF-44 specific complement mediated lysis of cultured $CD11c^+$ and $CD123^{hi}$ DC sort purified from a Lin^- preparation. **(A, B)** HLA-DR^+ $CD11c^+$ DC treated with autologous human serum (AS) and either (A) negative control IgM, or (B) CMRF-44 mAb. **(C, D)** HLA-DR^+ $CD123^{hi}$ DC

treated as in A, B. The same initial numbers of cells were treated in each case and the same number of TruCount beads were acquired for each dot plot.

Figure 6 are graphical representations showing the primary proliferative KLH response induced by PBMC is reduced by treatment with CMRF-44 and complement. Treatments = CMRF-44 mAb and AS, (shaded bars) or CMRF-44 and HIAS (Black bars). (* - $p<0.05$ Student's t-test, error bars ± 2SE). Two independent experiments **(A** and **B)** are shown.

Figure 7 are graphical representations showing recall proliferative response to tetanus toxoid (TT) induced by PBMC is reduced by treatment with CMRF-44 and complement. Treatments = CMRF-44 mAb and AS (shaded bars) or CMRF-44 and HIAS (black bars). (* - $p<0.05$ Student's *t*-test, error bars ± 2SE). Three independent experiments with different TT dose titrations **(A, B, C)** are shown.

Figure 8 are graphical representations showing CMRF-44 and complement treated PBMC stimulate a reduced allogeneic naïve $CD4^+$ T-lymphocyte reaction. Stimulators = irradiated overnight cultured PBMC treated with CMRF-44 and either AS (shaded bars) or HIAS (black bars). Responders = $CD4^+$ $CD45RA^+$ T-cells, 10^5/well. (* - $p<0.05$ Student's *t*-test, error bars ± 2SE). Two independent experiments **(A, B)** are shown.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is predicated in part on the observation that the activity of an APC such as, for example, a dendritic cell, can be suppressed *via* the specific targeting of an activation antigen with an effective down-regulatory agent. Moreover, a specific down-regulatory agent may preferentially target a distinct sub-population of APCs. The targeted APC is thereby disabled or destroyed, leading to the potentially negative effects of such cells being reduced or prevented. The identification of the capability to specifically down-regulate targeted APCs enables applications as diverse as removing or reducing the rejection difficulties caused by host *versus* graft and graft *versus* host incompatibility, and ameliorating a range of auto-immune inflammatory reactions characterized by unwanted immune responses such as, for example, rheumatoid arthritis.

Accordingly, one aspect of the present invention contemplates a method for modulating the immuno-activity of an APC, said method comprising contacting said APC with an effective amount of an agent, which agent couples, binds or otherwise associates with a cell-surface activation molecule for a time and under conditions sufficient to prevent, inhibit or otherwise down-regulate one or more functional activities of said APC.

Reference herein to an "antigen-presenting cell" or "antigen-presenting cells" or its abbreviation "APC" or "APCs" refers to a cell or cells capable of endocytotic adsorption, processing and presenting of an antigen. The term "antigen presenting" means the display of antigen as peptide fragments bound to MHC molecules, on the cell surface. Many different kinds of cells may function as APCs including, for example, macrophages, B cells, follicular dendritic cells and dendritic cells.

An "antigen" is any organic or inorganic molecule capable of stimulating an immune response. The term "antigen" as used herein extends to any molecule such as, but not limited, to a peptide, polypeptide, protein, nucleic acid molecule, carbohydrate molecule, organic or inorganic molecule capable of stimulating an immune response.

One particularly useful APC in the context of the present invention is a dendritic cell. Dendritic cells are a population of widely distributed leucocytes that are highly specialized in antigen presentation *via* MHC II antigen or peptide complexes. They are the principal activators of resting T cells *in vitro* and a major source of immunogenic epitopes for specific T cell clones following the detection of an antigen *in vivo* or *in vitro*. As used herein, the term "dendritic cell" or "dendritic cells" (DC) refers to a dendritic cell or cells in its broadest context and includes any DC that is capable of antigen presentation. The term includes all DC that initiate an immune response and/or present an antigen to T-lymphocytes and/or provide T-cells with any other activation signal required for stimulation of an immune response.

Accordingly, another aspect of the present invention contemplates a method for modulating the immuno-activity of a DC, said method comprising contacting said DC with an effective amount of an agent, which agent couples, binds or otherwise associates with a cell surface activation molecule, for a time and under conditions sufficient to prevent, inhibit or otherwise down-regulate one or more functional activities of said DC.

Reference herein to "DC" should be read as including reference to cells exhibiting dendritic cell morphology, phenotype or functional activity and to mutants or variants thereof. The morphological features of dendritic cells may include, but are not limited to, long cytoplasmic processes or large cells with multiple fine dendrites. Phenotypic characteristics may include, but are not limited to, expression of one or more of MHC class I molecules, MHC class II molecules, CD1, CD4, CD11c and CD123. Functional activity includes, but is not limited to, a stimulatory capacity for naive allogeneic T cells. "Variants" include, but are not limited to, cells exhibiting some but not all of the morphological or phenotypic features or functional activities of DC. "Mutants" include, but are not limited to, DC which are transgenic wherein said transgenic cells are engineered to express one or more genes such as genes encoding antigens, immune modulating agents or cytokines or receptors. Reference herein to a DC refers to both partially differentiated and fully differentiated DC and to activated and non-activated DC.

Without limiting the invention to any one theory or mode of action, two sub-populations of blood DC have been described, based on the differential expression of CD11c antigen and peanut agglutinin binding. They have distinctive characteristics and functions, including differential regulation by cytokines. The classical $CD11c^+$ "myeloid" DC traffic into tissues and mucosal surfaces to act as immune sentinel cells and, after activation by pathogens or appropriate inflammatory stimuli, migrate *via* lymphatics to secondary lymphoid organs, where they initiate immune responses. The $CD11c^-$ "lymphoid" DC express high levels of the CD123 antigen (interleukin-3 receptor α chain) on their surface. They are presumed to enter lymph nodes directly *via* the high endothelial venule to participate in immune responses. The $CD11c^+$ blood DC express the CD13 and CD33 myeloid differentiation antigens and include precursors for both epithelial and deep tissue (e.g. dermal) DC. In contrast, the $CD123^{hi}$ DC lack expression of CD13 and CD33 but